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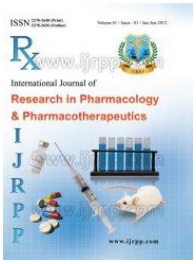

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Evaluation of anti-Parkinson's effect of *Peganum harmala* on haloperidol induced catalepsy in experimental animal model.

Abdulrahman M. Alshahrani *

[1]*Assistant Professor, College of Medicine, Shaqra University, Ministry of Higher Education, Kingdom of Saudi Arabia, Shaqra-11961.*

***Corresponding author: Abdulrahman M**

ABSTRACT

Aim of the study

To evaluate the neuroprotective activity of ethanolic extract of seeds of *Peganum harmala* (PHEE) in wistar rat.

Materials and methods

Ethanolic extract of seeds of *Peganum harmala* was screened for anti-Parkinson's effect by using behavioral method, haloperidol induced catalepsy at a dose of 50 mg/kg and 100 mg/kg. Distilled water and L-dopa were employed as control and standard groups respectively.

Results

Peganum harmala at a dose of 100 mg/kg showed maximal decrease in catalepsy (71±4.0 at 60 Minute) as compared to both negative and positive control groups.

Conclusion

Ethanolic extract of seeds of *Peganum harmala* may be useful in Parkinson's disease.

Keywords: Parkinson's disease, Haloperidol, *Peganum harmala*, L-dopa

INTRODUCTION

Parkinson's disease (PD) was first described by Dr. James Parkinson in a little book entitled An Essay on the Shaking Palsy, published in 1817. For the next century, the condition was known popularly as the shaking palsy and in the medical community by its latin equivalent, paralysis agitans. These terms are misleading, however, implying that people are paralyzed with this disorder, which is not the case. It is sometimes called idiopathic parkinsonism (the term idiopathic means that the cause is unknown), but more commonly today it is simply called Parkinson's disease, to honor the physician who first described it. PD is a disorder of the central nervous system, involving primarily a degeneration of certain nerve cells in deep parts of the brain called the basal ganglia, and in particular a loss of nerve cells (or neurons) in a part of the brainstem called the substantia nigra. These cells make the neurochemical messenger dopamine, which is partly responsible for starting a circuit of messages that coordinate normal movement. In the absence (or with substantial reduction, more than 80% of the normal level) of

dopamine, the neurons in the receiving area (called dopamine receptors) in the next part of the basal ganglia circuit called the striatum are not adequately stimulated, and the result is impairment of movement with tremor, slowness, stiffness, or balance problems.[1]

The ability of injected levodopa to improve akinesia in patients with PD was first demonstrated in 1961 and was followed by the development of oral levodopa later in the decade. [2,3] More recently, genetic mutations, abnormal handling of misfolded proteins by the ubiquitin–proteasome and the autophagy–lysosomal systems, increased oxidative stress, mitochondrial dysfunction, inflammation and other pathogenic mechanisms have been identified as contributing factors in the death of dopaminergic and non-dopaminergic cells in the brains of patients with PD. [4,5] There are four cardinal features of PD that can be grouped under the acronym TRAP: Tremor at rest, Rigidity, Akinesia (or bradykinesia) and Postural instability.[6] Bradykinesia refers to slowness of movement and is the most characteristic clinical feature of PD, although it may also be seen in other disorders, including depression. Bradykinesia is a hallmark of basal ganglia disorders [7-9]

In addition to rest tremor, many patients with PD also have postural tremor that is more prominent and disabling than rest tremor and may be the first manifestation of the disease [10, 11] Patients with PD may exhibit a number of secondary motor symptoms that may impact on their functioning at home, at work and while driving. [12] Because of a breakdown of the frontal lobe inhibitory mechanisms, some patients display a reemergence of primitive reflexes. [13, 14]

Harmal (Peganum harmala) is a plant of the family Zygophyllacea, native from the eastern Mediterranean region east to India. It is also known as Wild Rue or Syrian Rue because of its resemblance to plants of the rue family. It blossoms between June and August in the Northern Hemisphere. The flowers are white and are about 2.5–3.8 cm in diameter. The round seed capsules measure about 1–1.5 cm in diameter have three chambers and carry more than 50 seeds (Photograph 1 & 2). It is a perennial plant which can grow to about 0.8 m tall. [15] The Arabic Names for this herb are Ashqaqil, Aspand, Harmal.

Photograph 1: Seeds of *Peganum harmala*

Photograph 2: Seed Capsule of *Peganum harmala*

MATERIALS AND METHODS

Experimental animals

Wistar rat of male sex weighing 120–250 g were used. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethics Committee approved the experimental protocol. The animals were given standard diet. The animals had free access of standard diet and water and housed in a spacious cage for one week. rats were housed in cages of 5 at 22 ± 1◦C in a 12- h light/dark cycle. Tap water and food pellets were available as libitum. Groups of 6–11 rats were randomly assigned to different treatment groups and were tested in a counter balancing order. Animals were naive to experiment conditions. All experiments were carried out during night cycle of light and the experiments were carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals [16]. All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience [17]. The experimental protocol was approved by the Bioethical Committee on Animal Research. The minimum number of animals and duration of observations required to obtain consistent data were employed.

Drugs and Chemicals

Haloperidol (Serenace Injection), L-dopa (Syndopa, Sun Pharmaceuticals Ltd. Silvasa, India), Ethanol (Hi Media) propylene glycol (Hi Media) was purchased from the respective sources and was of analytical grade.

Treatment

The ethanolic extract of seeds of *Peganum harmala* was freshly dissolved in distilled water to be acutely administered to the rats. Doses of the extract and the time intervals were determined in preliminary tests. Negative control groups received only distilled water. All administrations were performed intraperitoneally (i.p.) in a dose volume of 1 ml/kg body weight. Thirty minutes after i.p. treatment, the animals were submitted to a battery of behavioral tests.

Source of *Peganum harmala* Seeds

Dried seeds of *Peganum harmala* were purchased from local market. The identity of the seed was confirmed by the Institutional Botanist. A voucher specimen (PAR-01) was kept in laboratory for future reference.

Preparation of Aqueous Extract

Dried seeds were homogenized to a fine powder. Hundred grams of powdered drug was Infused in 500 ml cold ethanol for 24 h, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. The extract was filtered, concentrated over the water bath and brought to dryness under vacuum. The yield of the extract was 8.7% (w/w).

Acute toxicity study

Acute toxicity study was performed using the limit test dose of 2000 mg/kg as described by Organization for Economic Cooperation and Development guideline and Interagency Research Animal Committee recommendation [18]. Six female rat were dosed sequentially and followed for any signs of toxicity and/ or death within 24 h and then for 14 days thereafter.

Haloperidol Induced Catalepsy in rat

Five Groups of 6 male Wistar rats with a body weight between 120 gm and 250 gm were used. They were dosed intraperitoneally with the test and standard drugs. Then , they were place individually into translucent plastic boxes with a wooden dowel mounted horizontally 10 cm from the floor and 4 cm from one end of the box. The floor of the box was covered with approximately 2 cm of bedding material. White noise was present during the test. Animals were allowed to adopt to the box for 2 min. then, each animal was grasped gently around the shoulders and under the forepaws and was placed carefully on the dowel. The standard (L-dopa) drug was administered by intraperitoneal route and test drug was administered by oral route, half an hour prior to the Haloperidol administration. The amount of time spend with at least one forepaws on the bar was determined. When the animal removes its paws, the time was recorded and rat was repositioned on the bar. Three trials was conducted for each animal at 30, 60, 120 and 360 min. The animal was considered to be cataleptic if it remains on the bar for 60 S. percentage of cataleptic animal was calculated.

Experimental groups

Negative Control group: rats were injected with distilled water.

Positive Control group: rats were injected with Haloperidol (1 mg/kg) intraperitoneally

Standard group: rats were injected with L-dopa (10 mg/kg) intraperitoneally.

Test group I: rats were injected with ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 50 mg/kg.

Test group II: rats were injected with ethanolic extract of seeds of *Peganum harmala* (PHEE) at dose of 100 mg/kg.

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULT

Acute toxicity test

At a single oral dose of 2000 mg/kg, seeds of *Peganum harmala* Ethanol Extract does not showed signs of toxicity or death in rats within the first 24 h and during the 14 days observation period.

Haloperidol Induced Catalepsy in rats

In the present study, the anti-Parkinson's effects of ethanolic extracts of seeds of *Peganum harmala* were evaluated using haloperidol induced catalepsy model in rats, in which PHEE at 50 mg/kg and 100 mg/kg affect the catalepsy score in dose dependant manner as compared to control and standard group. ethanolic extracts of seeds of *Peganum harmala* at a dose of 100 mg/kg showed maximal decrease in catalepsy at (76±3.0* at 30 min, 71±4.0* at 60 min, 92±5.0* at 120 min) found to be significant as compared to both negative and positive control groups as shown in table 1 and figure 1.

Table1: Effect of ethanolic extract of seeds of *Peganum harmala* on Haloperidol induced catalepsy in rats.

Treatment	Dose	Catalepsy Score (Second)			
		30 Min	60 Min	120 Min	360 Min
Distilled water (negative control)	-	15±2.5	16±3.1	15±1.5	16±3.2
Haloperidol (positive control)	1 mg/kg	149±4.1	158±2.2	177±5.1	120±3.5
Standard (L-dopa)	10 mg/kg	45±3.6	42±4.5	39±5.5	89±2.4
Test 1 PHEE	50 mg/kg	105±4.5	101±5.1	115±1.5	122±2.0
Test 2 PHEE	100 mg /kg	76±3.0*	71±4.0*	92±5.0	107±3.5

(The values are expressed in mean ±S.E.M. (n=6); $P < 0.05$)

Statistical analysis of data was performed with one way ANOVA.

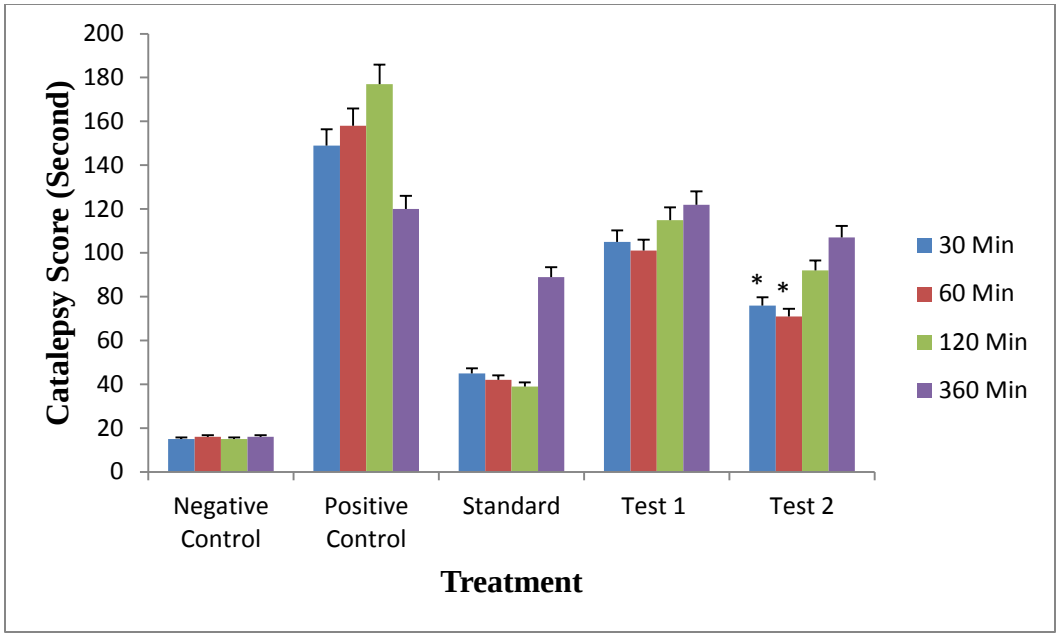

Figure 1: Effect of ethanolic extract of seeds of *Peganum harmala* on Haloperidol induced catalepsy in rats.

DISCUSSION

Animal models of are widely used to investigate its pathophysiological mechanisms and for exploring potential treatments. Typically, models of Parkinson's disease are characterized by measures of akinesia, such as in bar test for immobility. Neuroleptics such as haloperidol can produce a sustained but reversible akinesia, due to blockade of dopamine D2 receptors and this neuroleptic-induced Parkinsonism is a major side effect of their use in treatment of schizophrenia. D2 antagonists may act directly to reduce the ability of cortical and basal ganglia motor pathways to generate descending commands. Neuroleptics have thus been used as an acute model of Parkinson. The central dopaminergic function and evaluation of dopamine agonistic activity was carried out by observing the cataleptic behavior in rat. Haloperidol blocks the dopamine D2 receptors in the brain and precipitates the extra pyramidal side effects that can be measured by Bar test for catalepsy in rat. Oral movement is an important symptom presented by a series of neuropsychiatric conditions including Parkinson's disease. In addition, a spontaneous aging-induced oral dyskinesia has been extensively described. Thus, oral dyskinesia may represent the behavioral manifestation resulting from underlying mechanisms shared by different neuropsychiatric conditions. Tardive Dyskinesia is a motor side effect of long term treatment with typical neuroleptics (such as haloperidol) that involves involuntary movements of the face, mouth and tongue, but other different parts of the body may also be affected. Treatment with L-dopa (10 mg/kg, i.p.) significantly attenuated the dyskinetic behavior in rats. [21]

In the present study, the anti-Parkinson's effects of ethanolic extracts of seeds of *Peganum harmala* were evaluated using haloperidol induced catalepsy model in rats, in which PHEE at 50 mg/kg and 100 mg/kg affect the catalepsy score in dose dependant manner as compared to control and standard group. ethanolic extracts of seeds of *Peganum harmala* at a dose of 100 mg/kg showed maximal decrease in catalepsy as compared to both negative and positive control groups.

CONCLUSION

The present study investigated the putative effects of ethanolic extract of seeds of *Peganum harmala*. The extract may be useful in Parkinson's disease.

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Conflict of interest statement I declare that I have no conflict of interest.

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